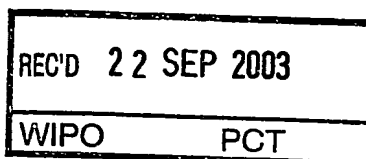


PCT/NZ03/00169



## CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 2 August 2002 with an application for Letters Patent number 520549 made by LINCOLN UNIVERSITY and RAVENSDOWN FERTILISER CO-OPERATIVE LIMITED.

Dated 10 September 2003.

## PRIORITY DOCUMENT

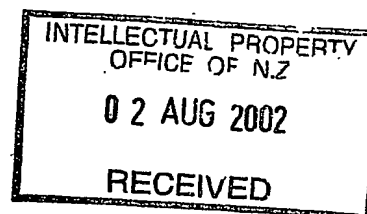
SUBMITTED OR TRANSMITTED IN  
COMPLIANCE WITH RULE 17.1(a) OR (b)

*Neville Harris*

Neville Harris  
Commissioner of Patents, Trade Marks and  
Designs



**BEST AVAILABLE COPY**



Patents Form No. 4

Our Ref: RL802499

Patents Act 1953  
PROVISIONAL SPECIFICATION

**Reductions in Nitrate Leaching, Reductions in Nitrous Oxide Emissions and  
Improved Pasture Production**

We, **LINCOLN UNIVERSITY**, incorporated under the Lincoln College Act 1961 as amended by virtue of the Education Amendment Act 1989, of Ellesmere Junction Road, Lincoln, Canterbury, New Zealand and **RAVENSDOWN FERTILISER CO-OPERATIVE LIMITED**, a New Zealand company, of Deloitte House, Level 1, 32 Oxford Terrace, Christchurch, New Zealand do hereby declare this invention to be described in the following statement:



**Reductions in Nitrate Leaching, Reductions in Nitrous Oxide Emissions and Improved  
Pasture Production**

**Technical Field**

5 This invention relates to reductions in nitrate leaching, reductions in nitrous oxide emissions and improved pasture production from grazed pasture soils. More particularly it relates to the method and delivery of a nitrification inhibitor, dicyandiamide (DCD) to grazed pasture soils.

10 **Background Art**

Nitrate ( $\text{NO}_3^-$ ) leaching from agricultural land and the contamination of ground- and surface-waters is a major environmental concern in many countries. This problem is particularly serious in intensive land use areas, where there are high inputs of nutrients in the forms of fertilizers or animal manure or effluents, or where nutrients  
15 are returned in the form of urine from the grazing animal.

High  $\text{NO}_3^-$ -N leaching losses (over  $100 \text{ kg NO}_3^- \text{ N ha}^{-1} \text{ y}^{-1}$  when urea fertiliser was applied at  $200 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ) have been reported on shallow stoney soils in Canterbury in the South Island of New Zealand. Studies in the UK on beef cattle grassland have  
20 recorded  $\text{NO}_3^-$ -N leaching losses ranging from 39 to  $162 \text{ kg N ha}^{-1} \text{ y}^{-1}$ .

In intensively grazed dairy pasture systems, the main source of  $\text{NO}_3^-$ -N leached comes from the nitrogen (N) returned in the urine from the grazing animal. The N loading rate

under a urine patch is equivalent to 1000 kg N ha<sup>-1</sup> and this amount of N is in excess of that which can be taken up by the pasture in a growing season. The surplus N, when converted to NO<sub>3</sub><sup>-</sup>, is thus prone to leaching when there is drainage through the soil profile.

5

The soil type has an influence on the amount of nitrate leached. On the plains of Canterbury, a number of new dairy farms have been, or are being, established on shallow stony soils. These soils cover more than 200,000 ha in the region. They only have a thin layer (usually no deeper than 30 cm) of fine material on the top, below which the soil contains high amounts of gravel.

10

Nitrate leaching from these free draining soils under flood-irrigated dairy farming conditions can be high. There is a general concern that significantly greater NO<sub>3</sub><sup>-</sup> leaching might occur after these soils are converted from dryland sheep farming to irrigated dairy farming.

15

The large dilution by drainage water from other sources (e.g. rivers, stock races and drainage from non-dairying areas) keeps NO<sub>3</sub><sup>-</sup>-N concentrations in the deep aquifer at present still very low. The expansion of dairy farming in the region requires improved farming practices to reduce the impact on groundwater quality by NO<sub>3</sub><sup>-</sup> leaching.

20

The New Zealand dairy industry has introduced its own environmental quality assurance programme for farmers to follow.

The New Zealand Department of Health has established drinking water guidelines that limit  $\text{NO}_3^-$ -N concentration in drinking water to  $11.3 \text{ mg N L}^{-1}$ . These practices are similar to regulatory or mitigating measures taken by other countries.

5

One of the management measures that has the potential to reduce both  $\text{NO}_3^-$  leaching and nitrous oxide emission from agricultural land is the use of nitrification inhibitors (NI) which slow down the conversion of ammonium ( $\text{NH}_4^+$ ) to  $\text{NO}_3^-$  in the soil.

10 Most soils in temperate regions of the world have a net negative charge, therefore  $\text{NH}_4^+$ -N is adsorbed onto the soil exchange surfaces, giving a greater opportunity for it to be taken up by plants, immobilized into soil organic matter, or fixed into certain clay mineral interlayers, rather than being leached.

15 Nitrous oxide ( $\text{N}_2\text{O}$ ) is both a greenhouse gas, contributing to global warming, and a gas that can cause depletion of the stratospheric ozone layer. The global warming potential of  $\text{N}_2\text{O}$  in the long-term is about 320 times that of carbon dioxide ( $\text{CO}_2$ ). The amount of  $\text{N}_2\text{O}$  directly emitted from agricultural fields may account for 20-30% of the total  $\text{N}_2\text{O}$  emitted annually from the earth's surface.

20

In grazed grassland systems, a major source of  $\text{N}_2\text{O}$  emissions is the N returned in animal excreta, particularly in the urine. For example, in New Zealand  $\text{N}_2\text{O}$  emissions from animal excreta account for about 50% of the country's total  $\text{N}_2\text{O}$  emissions.

Total N<sub>2</sub>O emissions make up about 20% of New Zealand's total greenhouse gas emissions inventory.

A significant reduction of N<sub>2</sub>O emissions from animal excreta in grazed pastures will therefore make a significant contribution to reducing total greenhouse gas emissions in New Zealand.

Pasture production under urine spots is higher than surrounding areas because of the N added to the soil by the urine although the efficiency of utilisation is not high.

It is an object of this invention to address the foregoing problems and to provide a useful alternative choice.

Further aspects and advantages of the present invention will become apparent from the ensuing description which is given by way of example only.

#### **Statements of invention**

The invention in a first aspect provides for the application of nitrification inhibitors in solution form as a very effective management tool to reduce NO<sub>3</sub><sup>-</sup>-N leaching, reduce nitrous oxide emissions and increase pasture production in the grazed pasture system.

The nitrification inhibitor may be applied in conjunction with irrigation water, by a spray vehicle or in a similar way to the application of other agricultural chemicals in liquid form.

The invention provides in another aspect a delivery mechanism for applying a nitrification inhibitor in solution form to a grazed pasture system.

5 The invention provides in a preferred aspect a solution of nitrification inhibitor (DCD) when applied at a frequency and timing to a grazed dairy pasture to reduce  $\text{NO}_3^-$ -N leaching by 76% for urine-N applied in the autumn, and by 42% for urine-N applied in the spring, giving an annual average reduction of 59%, which is equivalent to reducing the  $\text{NO}_3^-$ -N leaching loss in a grazed paddock from 118 to 46 kg N ha<sup>-1</sup> y<sup>-1</sup>.

10 Two of the most commonly used nitrification inhibitors are dicyandiamide (DCD) and nitropyrin. DCD has several advantages, which make it a desirable choice over others. It is cheaper to produce; it has a high water solubility and thus it can be applied in liquid form; and importantly, it decomposes completely in the soil into  $\text{NH}_4^+$  and  $\text{CO}_2$ .

15 The applicants postulate that as an alternative other nitrification inhibitors can be used such as 3,4-dimethylpyrazole phosphate (DMPP).

DCD can be regarded as a slow release N fertilizer (containing about 65% N), however this is not the purpose in the present invention of the proposed soil application. DCD inhibits the first stage of nitrification in soil, i.e., the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , by rendering the bacteria's enzymes ineffective. It is not a bactericide, and does not affect other heterotrophs that are responsible for much of the soil's biological activity. The applications of DCD in the past have been aimed at increasing the efficiency of N in fertilizers, or manures, or soil N released from mineralization.

25 However, DCD has not been used to specifically reduce  $\text{NO}_3^-$  leaching or nitrous oxide emissions from grazed pasture soils that receive animal urine returns.

The effective performance of DCD in reducing  $\text{NO}_3^-$ -N leaching and nitrous oxide emissions from urine patches compared to the performance reported from other studies, where DCD was either applied alone or combined with N fertilizers in cropping systems or applied with manure or dairy effluent, is related to the manner in which DCD is applied. When DCD is applied in solution form and between 5-9 times (following each urea and urine application) throughout a growing and grazing season it is highly effective at reducing nitrate leaching and nitrous oxide emissions.

- 10 The application in solution form helps the DCD to permeate throughout the soil surface layer enabling it to treat a greater soil volume, slowing down its decomposition compared to situations where it remains on the soil surface following application in a solid form with N fertilizer. Multiple applications maintain the inhibition effect in the soil for a longer time period compared to a single application.
- 15 Most other studies have either combined DCD with an N fertilizer applied in a solid form or mixed with a liquid manure or effluent in a single application.

- One of the keys to using nitrification inhibitors to reduce  $\text{NO}_3^-$  leaching and nitrous oxide emissions from urine patches is the delivery of the DCD over the entire soil surface, including the urine patches. This is achieved by applying DCD to the urine-affected paddocks as required (e.g. after each grazing rotation and urine deposition) in liquid form for example through the irrigation system, as described in the example method below.
- 20



Where farms are not irrigated, then the DCD solution can be applied by a spray vehicle in a similar way as agricultural chemicals (e.g. herbicides) are applied. An alternative means of delivering DCD is to apply DCD-urea mixtures in solution immediately following each grazing rotation.

Further aspects of the invention will become apparent from the following description which is given by way of example.

#### 10 Description of the drawings and tables

Figure 1. Shows total annual  $\text{NO}_3\text{-N}$  leached from the treatments as measured on the lysimeters, with and without a nitrification inhibitor (DCD).

Figure 2. Shows pasture yield as affected by the treatments measured on the lysimeters, with and without a nitrification inhibitor (DCD).

Figure 3. Shows pasture N offtake as affected by the treatments measured on the lysimeters, with and without a nitrification inhibitor (DCD).

Figure 4. Shows a nitrification inhibitor applied through a central pivot irrigation system.

20 Figure 5. Shows a nitrification inhibitor applied through a travelling irrigator.

Figure 6. Shows the active zone of the inhibitor, with and without irrigation.

Figure 7. Shows a pumping system for delivery of a nitrification inhibitor through an irrigation system.

Figure 8. Shows a nitrification inhibitor delivered by an agricultural spray vehicle.

Table 1. Shows a description of the treatments.

- 5 Table 2. Shows calculated paddock-averaged annual  $\text{NO}_3^-$ -N leaching losses and concentrations in the drainage water, with and without a nitrification inhibitor (DCD).

- 10 Table 3. Shows annual average pasture yield and N off-take calculated for a grazed paddock, with and without a nitrification inhibitor (DCD).

#### **Best Modes for carrying out the invention**

##### **Example 1**

- 15 Lysimeters studies, which are state-of-the-art technology for these investigations, have shown the effectiveness of a nitrification inhibitor, dicyandiamide (DCD), in reducing  $\text{NO}_3^-$ -N leaching from urine patches in a grazed dairy pasture under irrigation. This example uses a free-draining Lismore stony silt loam (Udic Haplustept loamy skeletal) and the pasture was a mixture of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) but the process is applicable to all temperate soils and animal grazing systems.
- 20

- Undisturbed soil monolith lysimeters, 50 cm diameter and 70 cm deep, were collected following well-established protocols and procedures that ensure there is minimal disturbance to the soil structure inside. The lysimeters were transported to a lysimeter facility near Lincoln University, using a specially designed trailer with air-bag suspension to minimize disturbance. The gap between the soil core and the metal casing was sealed using petroleum jelly to stop edge-flow effects. The lysimeters
- 25

were then installed in the field lysimeter facility with the surface of the lysimeters at the same level as that of the surrounding soil surface, in order to maintain normal plant growing conditions.

5 The treatments on the lysimeters are summarized in Table 1. Each treatment had 4 replicates. The treatments were allocated to the lysimeters in a randomised design. The urea application rate of  $200 \text{ kg N ha}^{-1} \text{ y}^{-1}$  was at the higher end of fertilizer N application rates for most farmers in the region. The rates of urea were either split into 8 applications to maximize N efficiency, or 4 applications as a cost cutting  
10 measure (i.e. reducing application costs). Urine was applied either in the spring (November) or in the autumn (April) to simulate urine patches deposited in the spring or autumn by a grazing dairy cow. The urine application rate of  $1000 \text{ kg N ha}^{-1}$  was equivalent to the typical N loading rate under a cow urine patch. The combination of urea with urine treatments was included to represent a situation where urea was  
15 applied throughout the paddock, and patches of the paddock received cow urine. Two rates of phosphate (P) fertilizers were applied,  $45$  or  $90 \text{ kg P ha}^{-1} \text{ y}^{-1}$ , to represent the range of P application rates used by local dairy farmers. As the purpose of this study was to determine the effectiveness of DCD in reducing  $\text{NO}_3^-$  leaching from cow urine, DCD was either applied or withheld in the paired urine-applied  
20 treatments (Table 1). The DCD was applied in a liquid form to simulate application in the irrigation water on irrigated dairy farms.

Urea was applied in solid form and was broadcast over the surface of the lysimeters followed by 10 mm of irrigation. Irrigation following urea application has been shown to significantly reduce ammonia loss by volatilization.

5 Fresh urine was collected early in the morning during the milking session from Friesian cows, and was analysed and applied to the lysimeters on the same day. The same volume of water was applied to the other lysimeters that did not receive urine in order to maintain the same moisture input to all lysimeters.

10 The P fertilizers were split into two applications, one in November and the other in March, following typical local farming practice. For the  $45 \text{ kg P ha}^{-1} \text{ y}^{-1}$  treatments, 20% potash superphosphate (containing 7% P, 10% K and 17% S) was used. For the  $90 \text{ kg P ha}^{-1} \text{ y}^{-1}$  treatments, a 50:50 blend of 20% potash superphosphate and triple superphosphate was used.

15

DCD was applied in solution form (100 mL per lysimeter) at the rates of  $7.5 \text{ kg ha}^{-1}$  after each urea application in the same day, or  $15 \text{ kg ha}^{-1}$  with each urine application. The DCD solution was sprayed onto the lysimeters with a watering can.

20 From November to April (late spring to mid autumn), flood irrigation, at 100 mm, was applied to all the lysimeters at about three weekly intervals. The amount of water applied was to represent the average amount of water applied on commercial flood-irrigated dairy farms. Irrigation water was applied using an electronically controlled

metering system to deliver the required volume of water to the lysimeters. From May to October (late autumn to mid spring), simulated rainfall was applied at the end of each month (if necessary), to supplement the natural rainfall received in order to equal the 75th percentile of local rainfall records for the same period of the year. This was done to create a so-called 'worst case scenario' in terms of rainfall inputs and to ensure a standard condition was achieved.

The herbage was cut periodically to simulate typical grazing practice. All the harvested herbage was removed and dry matter yield recorded. Herbage nitrogen content was analysed. Following each herbage cut, a specially designed mechanical cow hoof was used to simulate cow treading on the lysimeters. The mechanical hoof is made of stainless steel with identical shape and size as an adult Friesian cow hoof. The hoof is mounted onto a compressed air-ram, which is driven by an air compressor system to provide a treading pressure of 220 kPa to simulate the treading pressure exerted by a cow's hoof during walking. The entire surface of the lysimeters was trodden once following each herbage cut. This was based on the inventors observation of hoof print coverage following each grazing rotation.

Nitrous oxide emissions from two treatments (7 and 8 in Table 1) were determined using a closed chamber method. The enclosed chamber was fitted on top of the lysimeters inside a rubber ring on top of the lysimeter casing. At each sampling time, 3 samples, 10 minutes apart, were taken. Nitrous oxide was analysed using gas

chromatography and daily  $\text{N}_2\text{O}$  fluxes were calculated based on daily mean temperatures.

Table 1. Description of treatments.

| 5  | Treatment<br>No. | Urea<br>( $\text{kg N ha}^{-1} \text{ y}^{-1}$ ) | Urine         | P fertilizer<br>( $\text{kg P ha}^{-1} \text{ y}^{-1}$ ) | DCD |
|----|------------------|--|---------------|--|-----|
|    | 1 (Control)      | 0  | 0             | 45   | No  |
|    | 2                | 200/8  | 0             | 45   | No  |
| 10 | 3                | 200/8  | 1000 (Autumn) | 45   | No  |
|    | 4                | 200/8  | 1000 (Autumn) | 45   | Yes |
|    | 5                | 200/8  | 1000 (Autumn) | 90   | No  |
|    | 6                | 200/8  | 1000 (Autumn) | 90   | Yes |
|    | 7                | 200/4  | 1000 (Spring) | 45   | No  |
| 15 | 8                | 200/4  | 1000 (Spring) | 45   | Yes |

The urea rate was split into 8 (200/8) or 4 (200/4) applications. The urine was applied in one application, in either Spring (November) or Autumn (April). The P fertilizers were split into two applications, one in November and the other in March. For the  $45 \text{ kg P ha}^{-1} \text{ y}^{-1}$  treatment, 20% potash superphosphate was used. For the  $90 \text{ kg P ha}^{-1} \text{ y}^{-1}$  treatment, a 50:50 blend of 20% potash superphosphate and triple superphosphate was used. DCD was applied in solution form (100 mL per lysimeter) at the rates of  $7.5 \text{ kg ha}^{-1}$  after each urea application in the same day, or  $15 \text{ kg ha}^{-1}$  with each urine application.

Table 2. Calculated paddock-averaged annual  $\text{NO}_3^-$ -N leaching losses and concentrations in the drainage water, with and without a nitrification inhibitor (DCD).

| Management condition                  | Leaching losses from paddock <sup>c</sup> (kg N ha <sup>-1</sup> y <sup>-1</sup> ) | Paddock averaged concentration (mg N L <sup>-1</sup> ) |
|---------------------------------------|--|--|
| Urea 200 + Grazing <sup>a</sup>       | 118.2  | 19.7   |
| Urea 200 + grazing + DCD <sup>b</sup> | 46.1   | 7.7  |

<sup>a</sup> Assuming that urea was applied at 200 kg N ha<sup>-1</sup> y<sup>-1</sup> throughout the paddock, and urine patches covered 25% of the paddock (equivalent to 3 cows per ha.).

<sup>b</sup> As above except with DCD applied.

<sup>c</sup> To calculate these values, annual leaching losses from urine areas were the averages of those from the urine applied in the spring and autumn. The autumn leaching losses were the averages of the P45 and P90 treatments. The leaching losses from the urea 200 treatment with DCD were assumed to be 59.1% less than that from the urea 200 treatment without DCD (the average reduction in leaching loss in urine areas) (there was not a urea + DCD treatment in this study).

Table 3. Annual average pasture yield and N off-take calculated for a grazed paddock, with and without a nitrification inhibitor (DCD).

| Management condition                  | Pasture yield (t ha <sup>-1</sup> y <sup>-1</sup> ) | N off-take (kg N ha <sup>-1</sup> y <sup>-1</sup> ) |
|---------------------------------------|---|---|
| Urea 200 + grazing <sup>a</sup>       | 11.4  | 351.7   |
| Urea 200 + grazing + DCD <sup>b</sup> | 15.0  | 406.3   |

<sup>a, b</sup> Similar assumptions were made as those for calculating  $\text{NO}_3^-$ -N leaching losses in Table 2.

**Example 2****Delivery Systems for applying a Nitrification Inhibitor through an Irrigation System**

5 The concept is to spread nitrification inhibitor evenly over the pasture soil by applying it through an irrigation system (such as central pivot or travelling irrigator) where there is an ability to control and vary the application volume and rate of application according to the conditions in the soil (as illustrated in Figs 4 and 5).

10 Delivery of the inhibitor in solution and mixed with the irrigation water ensures that the inhibitor penetrates throughout the soil surface. The ability to control the penetration of the inhibitor through the soil volume is a key determinant of the effectiveness of the compound and is central to the uniqueness of this process. The  
15 effectiveness of the inhibitor is increased by distribution throughout, and thus treats, a larger volume of soil (Fig. 6) than it would if the inhibitor alone was sprayed onto the pasture soil surface. This is an advantage of such a delivery system where the soil moisture content varies from wilting point to field capacity throughout the year and in turn affects the flow of the applied solution.

20 A computer controlled pumping system may be part of the inhibitor delivery system. The nitrification inhibitor solution at a concentration dependent on the level of control required over the processes in the soil is injected from a supply tank into the irrigation water using a flow rate controlled pump connected to the irrigation delivery pipe or  
25 irrigation hose (as illustrated in Fig. 7).

The timing of application is important to the success of the process. The irrigator and pumping systems are controlled such that the nitrification inhibitor is applied to the paddock soon after grazing, when the urine patches are fresh, by following the  
30 grazing rotation. This is particularly important for the autumn grazing rotations. The ability to control the lag time between urine deposition and treatment application determines the extent of nitrate production in the soil and the subsequent amount



leached into the groundwater and aquifers that are the principal concern of this treatment method.

**Example 3****Delivery of a nitrification inhibitor using agricultural chemical spray equipment**

- 5 The nitrification inhibitor can be delivered evenly over the soil surface using agricultural chemical spray equipment (e.g., equipment currently used to apply agricultural chemicals such as herbicides or pesticides).

10 The nitrification inhibitor is delivered/dissolved in water and the solution is sprayed onto the soil from the tank of an agricultural spray vehicle.

The spray equipment can be used to apply the nitrification inhibitor immediately after grazing when the animal urine patches are 'fresh', this can be particularly effective during autumn grazing rotations.

- 15 Following the spray application by this method irrigation water can be applied to 'wash' the nitrification inhibitor into the topsoil. This will ensure that the nitrification inhibitor is distributed evenly throughout, and thus treats, the topsoil.

- 20 If irrigation is not applied then the nitrification inhibitor can be applied immediately prior to rainfall. The rain will 'wash' the inhibitor into the topsoil and thus ensure that this larger volume of soil is treated.

**Advantages**

- 25 The invention provides a number of advantages some of which are listed below.

1. The  $\text{NO}_3\text{-N}$  concentration in the drainage water is reduced accordingly from 19.7 to 7.7 mg N  $\text{L}^{-1}$ , with the latter being below the World Health Organisation and  
30 New Zealand Department of Health drinking water guideline of 11.3 mg N  $\text{L}^{-1}$  (Table 2).

2. A solution of nitrification inhibitor (DCD) when applied at a frequency and timing to a grazed dairy pasture increases pasture production by more than 30%, from 11.4 to 15.0 t ha<sup>-1</sup> y<sup>-1</sup>.

3. Total annual NO<sub>3</sub><sup>-</sup>-N leaching losses measured from the lysimeters were low in the Control (4.8 kg N ha<sup>-1</sup> y<sup>-1</sup>) and in the Urea 200 treatment (Treatment 2: 7.9 kg N ha<sup>-1</sup> y<sup>-1</sup>) (Figure 1). In Treatment 3 where urine was applied in the autumn without DCD, total NO<sub>3</sub><sup>-</sup>-N leaching loss was equivalent to 516 kg N ha<sup>-1</sup> y<sup>-1</sup> (i.e. directly below the urine patch). This was reduced to 128 kg N ha<sup>-1</sup> y<sup>-1</sup> when DCD was applied (Treatment 4) (Figure 1).

4. Similarly, in the P 90 treatments (cf. Treatments 5 and 6), the application of DCD reduced total annual NO<sub>3</sub><sup>-</sup>-N leaching loss from 488 to 112 kg N ha<sup>-1</sup> y<sup>-1</sup>. Where the urine was applied in the spring (cf. Treatments 7 and 8), the application of DCD reduced total annual NO<sub>3</sub><sup>-</sup>-N leaching loss from 397 to 230 kg N ha<sup>-1</sup> y<sup>-1</sup> (Figure 1). These results show that the application of DCD reduced NO<sub>3</sub><sup>-</sup>-N leaching by an average of 76.1% for the urine-N applied in the autumn, and by 42.1% for the urine N applied in the spring.

5. When urea was applied at 200 kg N ha<sup>-1</sup> y<sup>-1</sup> throughout the paddock and the paddock was grazed by 3 cows per ha, the average annual NO<sub>3</sub><sup>-</sup>-N leaching loss from the paddock was estimated to be 118 kg N ha<sup>-1</sup> y<sup>-1</sup> without DCD. This was reduced to 46 kg N ha<sup>-1</sup> y<sup>-1</sup> (Table 2) when DCD was applied. The application of DCD would reduce the NO<sub>3</sub><sup>-</sup>-N concentration in the drainage water from 19.7 to 7.7 mg N L<sup>-1</sup>. The latter was below the drinking water guideline of 11.3 mg N L<sup>-1</sup> set by the New Zealand Department of Health. Most of the NO<sub>3</sub><sup>-</sup>-N leached was contributed by that leached from the urine patch areas (Table 2).

6. The application of DCD increases pasture yields (Figure 2). The increases in pasture yields were particularly evident in the autumn urine treatments (cf. Treatments 3 and 4; and 5 and 6). The application of DCD increased pasture yields in the autumn urine treatments by an average of 49%, while the increase in the spring urine treatments was equivalent to 17.5%. This gave an annual average increase of 33.3% in pasture production by the application of DCD. The pasture yields were higher in the spring urine treatments than in the autumn urine treatments.

7. The increases in pasture N off-take as a result of DCD application were equivalent to 23% for the autumn urine treatments, and 9% for the spring urine treatments, giving an annual average of 16%.

8. Similar calculations were carried out for average paddock pasture yield and N off-take as for  $\text{NO}_3^-$ -N leaching losses by substituting pasture yield or N off-take for  $\text{NO}_3^-$ -N leached (Table 4). Without DCD, the average annual pasture yield in the grazed paddock was  $11.4 \text{ t ha}^{-1} \text{ y}^{-1}$ , and the N off-take was  $352 \text{ kg N ha}^{-1} \text{ y}^{-1}$ . These were increased to  $15 \text{ t ha}^{-1} \text{ y}^{-1}$  and  $406 \text{ kg N ha}^{-1} \text{ y}^{-1}$ , respectively, when DCD was applied (Table 3).

9. The application of DCD applied in solution form could potentially be a very effective management tool to reduce  $\text{NO}_3^-$ -N leaching in a grazed pasture system. The use of DCD alone could reduce  $\text{NO}_3^-$ -N concentration in the drainage water from 19.7 to 7.7  $\text{mg N L}^{-1}$  in a shallow and stony free-draining Lismore soil which is a soil that has a very high leaching potential. Therefore, with the use of DCD, the  $\text{NO}_3^-$ -N concentration in the drainage water from free-draining soils can be reduced significantly below the drinking water guideline of 11.3  $\text{mg N L}^{-1}$ .

10. For instance, combined DCD with farm dairy effluent (dirty water) reported only an 18% reduction in the amount of  $\text{NO}_3^-$ -N leached. This was significantly lower

than the 42.1-76.1% reductions in the amount of urine N leached as measured in this study.

11. Any additional costs would be small compared with the enormous cost that would be required to clean up a badly contaminated aquifer. Further work may be required to develop an injection system to incorporate the DCD into the irrigation system, similar to a fertigation system, and to conduct field evaluation of performance.

12. The rate of DCD decomposition increases with temperature. This is probably one of the reasons why the reduction in  $\text{NO}_3^-$  leaching from the spring urine application was smaller than that from the autumn urine application, as the DCD would have been decomposed more rapidly under the warmer summer temperatures. However, the fact that DCD was only applied 5 times in the spring urine treatment compared to 9 applications in the autumn urine treatments might also have played a part in contributing to the differences in the DCD effect. In line with findings in a previous study using the same soil the amount of  $\text{NO}_3^-$ -N leached from the spring urine without DCD was lower than that from the autumn urine partly due to greater pasture N off-take of the spring urine N (Figure 7). The optimum frequency and amount of DCD application are subjects of the inventors current investigation.

13. The use of DCD increases pasture production (Figures 2 & 3, Table 3). The slow down of nitrification therefore preserves the N in the  $\text{NH}_4^+$  form for a longer period, making it more accessible for pasture uptake. This was in agreement with a number of previous studies that showed that DCD could increase the efficiency of N fertilizers, or manures. This increase in pasture production is an added benefit from applying DCD onto grazed pastures. The additional amount of N applied from the DCD was small ( $29\text{-}49 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ), particularly compared with the  $1000 \text{ kg ha}^{-1}$  of urine N applied, the effect on pasture production by the DCD-N alone was, therefore, probably limited.

14. The amount of  $\text{NO}_3^-$ -N leached from the Urea 200 treatment ( $8 \text{ kg N ha}^{-1} \text{ y}^{-1}$ , Treatment 2, Figure 1) was slightly lower than that ( $17 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ) from a previous study using the same soil. This difference in leaching loss was probably because the  
5 200 kg of urea N in this study was split into 8 applications whereas in the previous study, it was split into 4 applications. The practice of more frequent applications of smaller amounts of N was thus probably slightly more efficient than that of fewer applications at greater amounts.

10 15. Treating the soil with DCD in a grazed pasture, including the urine patches, the amount of  $\text{NO}_3^-$  leaching can be dramatically reduced. The use of DCD reduced  $\text{NO}_3^-$ -N leaching by 76% for the urine N applied in the autumn, and by 42% for urine N applied in the spring, giving an annual average reduction of 59%. This was found to reduce the  $\text{NO}_3^-$ -N leaching loss in a grazed paddock from 118 to 46  $\text{kg N ha}^{-1} \text{ y}^{-1}$ .

15 16. The  $\text{NO}_3^-$ -N concentration in the drainage water was reduced accordingly from 19.7 to 7.7  $\text{mg N L}^{-1}$ , with the latter being below the drinking water guideline of 11.3  $\text{mg N L}^{-1}$ . In addition to the environmental benefits, the use of DCD also increased pasture production by more than 30%, from 11.4 to 15.0  $\text{t ha}^{-1} \text{ y}^{-1}$ .

20 17. Total  $\text{N}_2\text{O}$  emissions following two urine applications were reduced from 46  $\text{kg N}_2\text{O-N ha}^{-1}$  without DCD to 8.5  $\text{kg N}_2\text{O-N}$  with DCD, representing an 82% reduction.

25 18. These results suggest that the effective use of DCD has the potential to transform grazed pasture systems to more environmentally sustainable basis by reducing  $\text{NO}_3^-$  leaching and  $\text{N}_2\text{O-N}$  losses.

19. The delivery system for applying an active nitrification inhibitor through an irrigation system can produce the effects described above.

20. The delivery system for applying an active nitrification inhibitor using agricultural chemical spray equipment.

Where in the description and examples particular integers are mentioned it is envisaged that their equivalents may be substituted as if they were individually set forth herein.

Particular examples of the invention have been described and it is envisaged that improvements and modifications can take place without departing from the scope of the invention.

LINCOLN UNIVERSITY and  
RAVENSDown FERTILISER  
CO-OPERATIVE LIMITED

By their Attorneys

BALDWIN SHESLTON WATERS

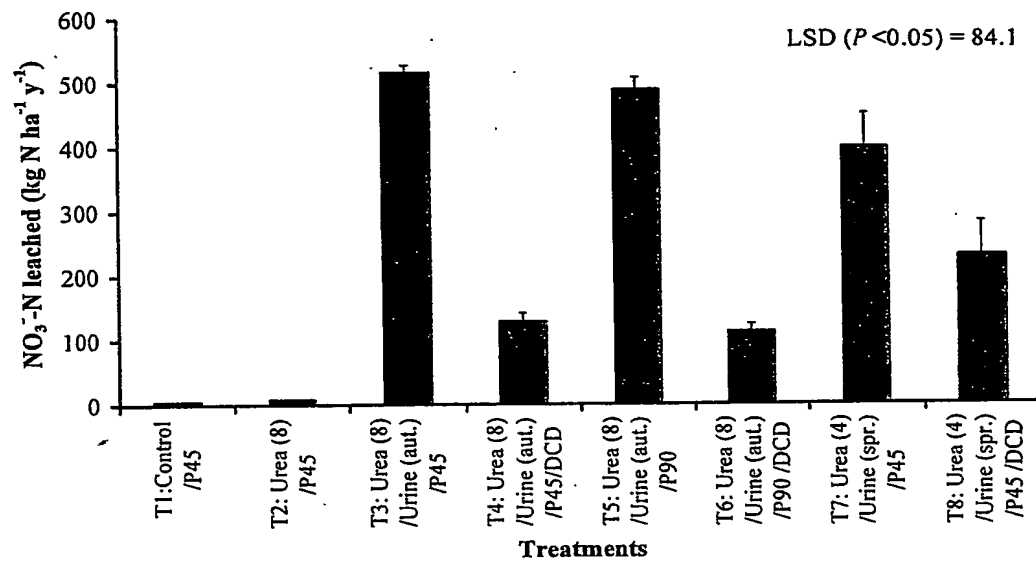


Figure 1. Total annual  $\text{NO}_3^-$ -N leached from the treatments as measured on the lysimeters, with and without a nitrification inhibitor (DCD).



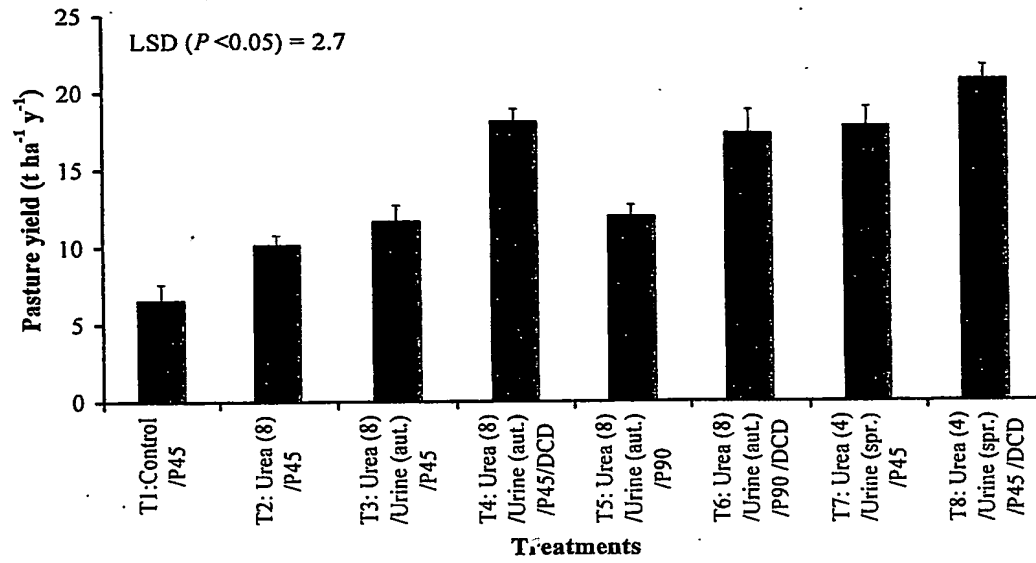


Figure 2. Pasture yield as affected by the treatments measured on the lysimeters, with and without a nitrification inhibitor (DCD).

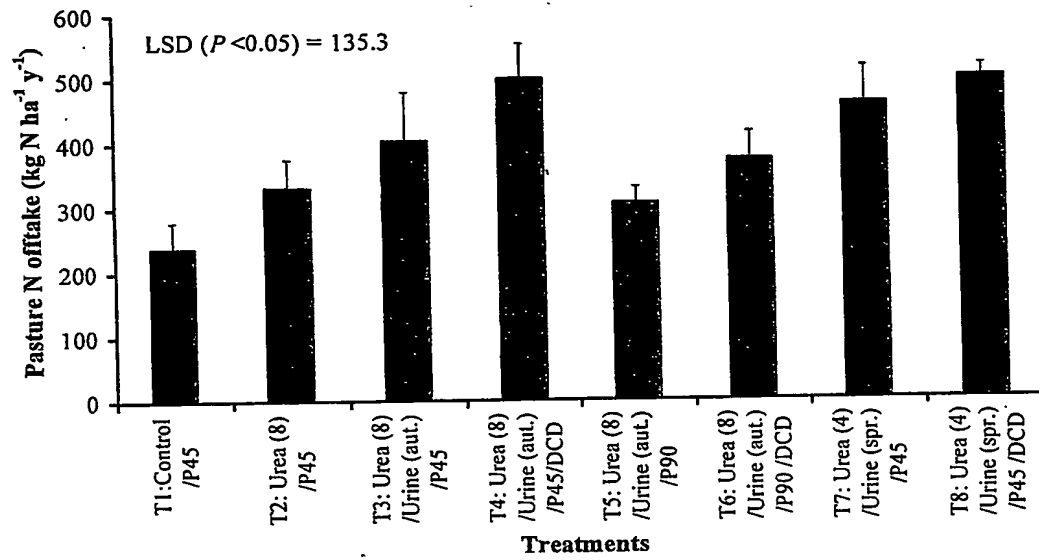


Figure 3. Pasture N off-take as affected by the treatments measured on the lysimeters, with and without a nitrification inhibitor (DCD).

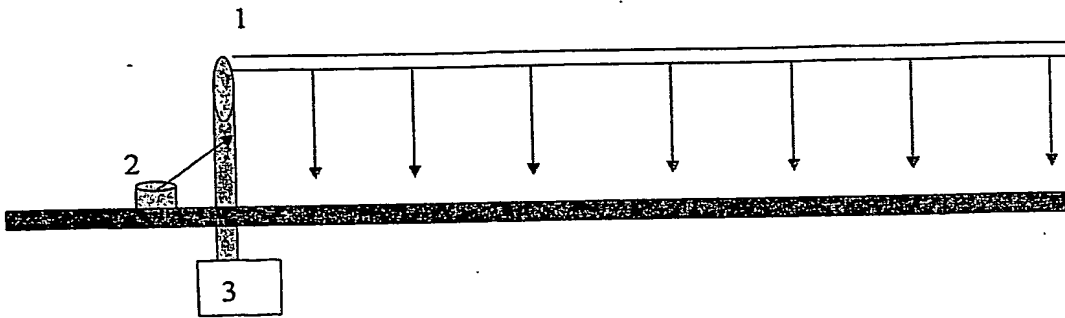


Figure 4. Nitrification inhibitor applied through a central pivot irrigation system

Legend

- 1= Centre pivot irrigation system
- 2= Supply of nitrification inhibitor into irrigation water (by Venturi pipe or pump)
- 3= Water supply

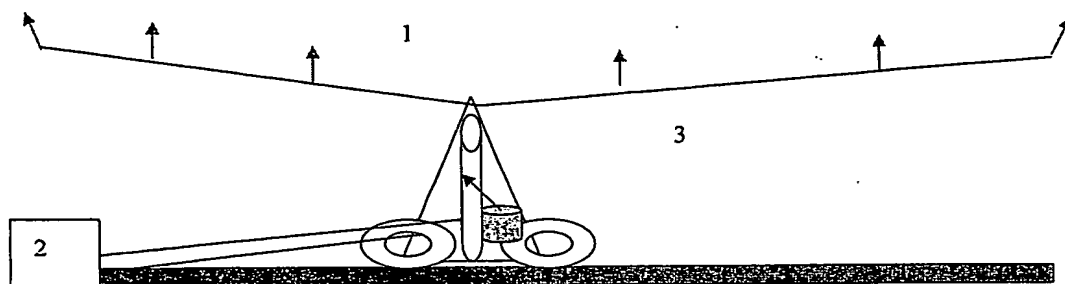


Figure 5. Nitrification inhibitor applied through a travelling irrigator

Legend

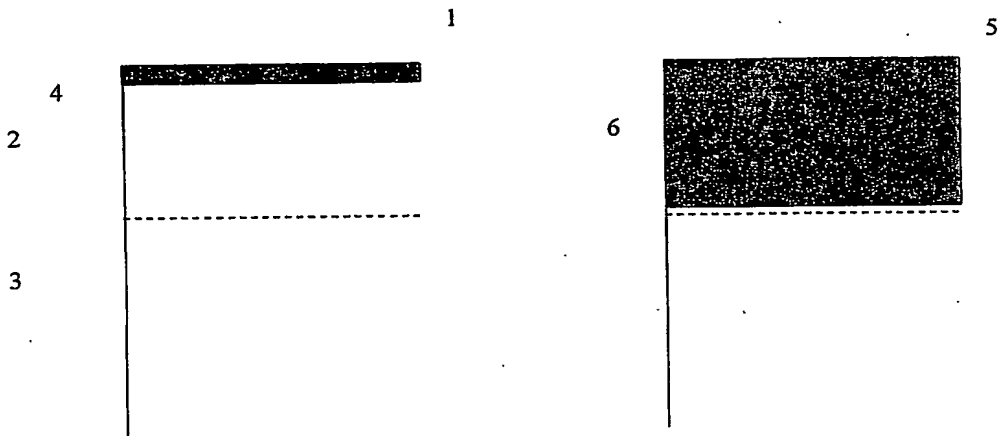
1= Travelling irrigator

2= Water supply

3= Supply of nitrification inhibitor into irrigation water (by Venturi pipe or pump)

Inhibitor applied to soil alone

Inhibitor applied to soil  
along with irrigation water



Greater volume  
treated

Figure 6. Active soil zone of the nitrification inhibitor, with and without irrigation

Legend

- 1= Soil surface
- 2= Topsoil
- 3= Sub-soil
- 4= Small volume of soil treated
- 5= Soil surface
- 6= Greater volume of soil treated

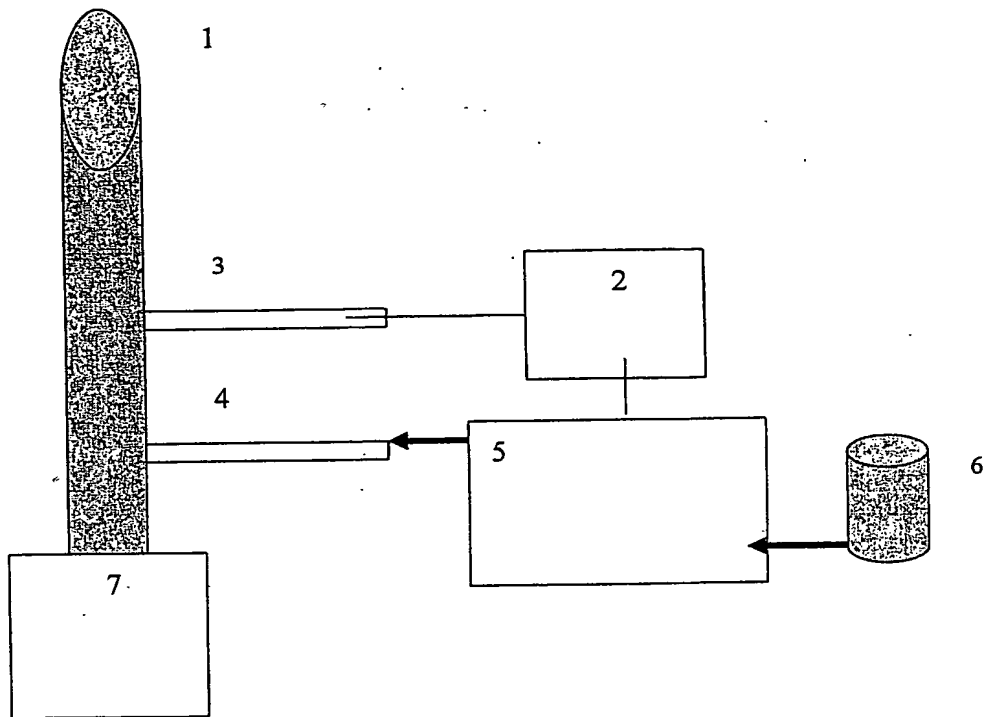


Figure 7. Pumping System for delivery of a nitrification inhibitor through an irrigation system

Legend

- 1= Irrigation water plus nitrification inhibitor delivered at controlled rate to irrigator
- 2= Control system
- 3= Flow rate sensor
- 4= Control valve
- 5= Injection pump
- 6= Supply of nitrification inhibitor
- 7= Irrigation pump

8/8

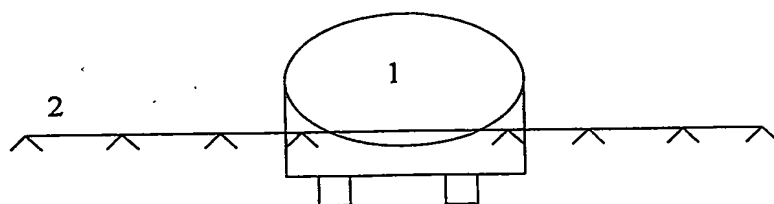


Figure 8. Nitrification inhibitor delivered by an agricultural spray vehicle

Legend

- 1= Vehicle with tank containing nitrification inhibitor solution
- 2= Spray boom to apply inhibitor over the soil surface

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**